NCI Translates: The NCI Translational Science Meeting

November 7-9, 2008
Washington, D.C.

Meeting Summary

Despite major advances in cancer biology over recent years, current translational systems have been largely unable to convert these discoveries into concrete clinical applications in a timely manner. On November 7-9, 2008, the National Cancer Institute (NCI) hosted nearly 800 researchers, patient advocates, and government officials at *NCI Translates: The NCI Translational Science Meeting* as part of a concerted effort to accelerate translational research.

Purpose and Goals

The overall purpose of the Translational Science Meeting was to accelerate translational research. The meeting was specifically organized to:

- showcase NCI-supported translational research (through abstract submissions and poster presentations),
- expand the range of collaborations and interactions between investigators across all NCI funding mechanisms,
- familiarize the translational cancer research community with the Translational Research Working Group (TRWG) Developmental Pathways to Clinical Goals,
- discuss specific examples of ñTranslational Research Opportunitiesò and the information necessary to evaluate and prioritize translational research projects, and
- demonstrate that there are compelling ñTranslational Research Opportunitiesò warranting acceleration.

Posters presented and ñTranslational Research Opportunity: Information Guidesò developed at the meeting will be used by NCI to assess the breadth and quality of its translational research portfolio and to determine whether prioritization of translational research opportunities is warranted and feasible. Subsequent steps will include the design and implementation of a prioritization process as well as development of funding mechanism(s) and project management services to support acceleration of prioritized projects.

Opening Joint Session—Setting the Stage

The Translational Research Working Group (TRWG)

The TRWG was a NCI-sponsored working group charged with evaluating the status of NCIôs investment in translational research and envisioning its future in an inclusive, representative, and transparent manner. In 2007, the TRWG presented a report entitled *Transforming Translation: Harnessing Discovery for Patient and Public Benefit* to the National Cancer Advisory Board (NCAB), which accepted the 15 recommendations proposed to accelerate translational cancer research (http://www.cancer.gov/trwg). Implementation of the TRWG recommendations is being carried out by the Coordinating Center for Clinical Trials (CCCT) in collaboration with NCIôs Divisions, Offices, and Centers. The CCCT was initially created to oversee the implementation of changes to the NCI clinical trials system as recommended by the Clinical Trials Working

Group (CTWG). Integration of CTWG- and TRWG-related activities will ensure that the continuum between translational and clinical research is as seamless as possible.

The TRWG concluded that basic science research is best driven by the ingenuity of individual investigators and that translational research would benefit from dedicated management. This is in part due to the fact that translational research requires an alignment of several research domains in pursuit of a specific, clinically relevant goal. As a result, emphasis has been placed on the TRWG recommendations that call for the establishment of a yearly process to identify a small number of projects that are ñripeò for translation as well as for provision of the financial resources and project management support required to expedite these projects to the point of early-phase clinical trials. The envisioned prioritization process is designed to build upon the successes of current NCI-funded translational research programs, not replace them.

The TRWG Developmental Pathways to Clinical Goals

The TRWG developed six Developmental Pathways to Clinical Goals, which are process diagrams that resemble engineering flow charts and outline the steps required to advance a basic science discovery to early-phase clinical trials. Two of the TRWG Pathways focus on the development of assessment tools (Biospecimen- and Imaging-based Assessment Modalities), while the remaining four focus on development of interventions for cancer treatment or prevention (Agents, Immune Response Modifiers, Interventive Devices, and Lifestyle Alterations). Each Pathway has five domains representing the progression of translational research: 1) credentialing, 2) creation of the modality, 3) supporting tools, 4) preclinical development, and 5) early-stage clinical trials. The TRWG Pathways provide a framework for identifying the requisite experience, resources, and capabilities to move credentialed basic, clinical, or population science discoveries forward to the point where definitive late-stage clinical trials might be warranted.

The Pathways are expected to serve as useful tools for the research community, allowing individual investigators/programs focused on one aspect of a translational research question to consider their work within a broader developmental context, prompting them to develop the collaborations necessary to move their research forward. To help familiarize meeting attendees with the Pathways, former TRWG members provided concrete examples showing how translational research projects could be mapped to a Pathway. One presentation tracked the development of a successful drug through the steps of the Agent Pathway; the other followed a biomarker still under development through the early stages of the Biospecimen-based Assessment Modalities Pathway and discussed how the opportunity could be advanced.

The Pathways, which are described in detail in *Clinical Cancer Research* 14: 5663-5713, 2008, have helped NCI articulate two broad goals for optimizing translational research: 1) To assure that the most promising translational research concepts enter a developmental Pathway in a timely manner; and 2) To advance these concepts to the clinic or to a productive failure as rapidly and efficiently as possible.

Identifying Translational Research Opportunities

NCI is also interested in using the Pathways to Clinical Goals to facilitate prioritization of ñTranslational Research Opportunities.ò NCI recognizes that creation of a robust prioritization process will require development of new tools to identify projects worthy of targeted investment. In this regard, ñTranslational Research Opportunity: Information Guidesò were drafted based on each of the six TRWG Pathways. These guides provided a structured format for gathering information on opportunities in translational cancer research that would benefit from focused funding and dedicated project management. Information collected includes the strength of the

projectôs scientific rationale, clinical or public health importance, technical feasibility of the envisioned development approach, and suitability for NCI investment. NCI used the Translational Science Meeting as a venue to pilot and collect feedback on the information guides. This was done as part of the small group poster presentations and discussions.

Concurrent Poster Viewing and Discussion Sessions—Mapping Translational Research to the Pathways to Clinical Goals

Prior to the NCI Translational Science Meeting, 87 NCI Program Directors from seven Divisions, Offices, and Centers identified translational research grants within their portfolios. Investigators associated with these grants were invited to submit abstracts for presentation at the meeting. More than 500 submitted abstracts were coded to one or more of the Pathways to Clinical Goals as well as to a population(s) and organ site(s). The Program Committee, comprised of 22 intramural and extramural translational researchers, organized the abstracts into the following 25 poster discussion sessions based on Pathway as well as scientific or clinical focus:

Agents

- Biochemical Targets and Drug Screening
- Stem Cells, Gene Expression, and Epigenetics
- Drug Delivery and Gene Therapy
- Integrative Biology
- Prostate Cancer
- Pancreatic and Breast Cancers
- Hematological Malignancies
- Head, Neck, and Lung Cancers

Biospecimen-Based Assessment Modalities

- ñ-Omicò Technologies
- Prognostic and Predictive
- Early Detection
- Breast Cancer
- Prostate and Bladder Cancers
- Esophagus, Colon, and Liver Cancers
- Lung Cancer
- Hematological and Pediatric Cancers

Imaging-Based Assessment Modalities

- Approaches to Cancer Detection
- Imaging and Cancer Therapeutics

Immune Response Modifiers

- Antibodies, Cytokines, and Viruses
- Cancer Vaccines
- Cellular Therapies

Interventive Devices

- Ionizing and Non-ionizing Radiation
- Devices for Surgical Ablation and Biopsy

Lifestyle Alterations

- Dietary Components
- Biobehavioral Mechanisms

The 25 poster sessions were divided into three concurrent sessions. For each poster session, the first hour was allotted for poster viewing. During this time, investigators had the opportunity to discuss their research and network with other researchers. Meeting participants were encouraged to not only learn of their colleaguesô work but also to identify opportunities for collaboration that may help advance their translational research projects toward a clinical goal.

Following the poster viewing, a formal discussion was convened in each session to allow participants to jointly consider how research from the session could coalesce into a ñTranslational Research Opportunityò along a specific Pathway by presenting specific examples.

Each discussion was led by two scientific co-chairs with an NCI representative available for facilitation. Two patient advocate co-chairs were also present in each session to ensure that the patient perspective was represented. In addition to discussing specific research projects, session participants commented on the value of the Pathways for organizing translational research in a milestone-driven manner, identifying common ñbottlenecks,ò and elucidating domains that may benefit from additional investment (e.g., GMP/GLP).

Closing Joint Session—Translational Research Opportunity Examples

NCI will use the ñTranslational Research Opportunityò examples generated in the poster discussion sessions to determine whether the information outlined in the ñTranslational Research Opportunity: Information Guidesò would be sufficient to identify potential candidates for prioritization. During the closing joint session, scientific co-chairs from various sessions presented one example of a potential ñTranslational Research Opportunityò related to each of the 6 Pathways to Clinical Goals. The specific examples are detailed in the Appendix of this meeting summary.

Cancer Translational Research—An Engineer's Point of View

Dr. David Dilts, Professor in the Schools of Engineering and Management at Vanderbilt University, reminded meeting attendees that there will never be enough funding or resources to support the wealth of translational research ideas that exist, particularly in these troubled economic times. He emphasized that cancer research must compete with many other societal needs for public funds, making the prioritization of research a critical step in the process to ensure that available resources are being optimally used. This means that some projects, including some excellent projects, will not be funded. He warned against feeling entitled to funding and encouraged the translational research community to use the TRWG Pathways to Clinical Goals to assist in the organization of research efforts, elimination of inefficiencies, and completion of projects, thus, demonstrating the value of translational cancer research to consumers. However, he also remarked that the philosophy that led to the development of the Pathways is more important than the tools themselves; thus, the Pathways should only be used to the extent that they facilitate research and should be modified as necessary.

Summary and Next Steps

The goals of the NCI Translational Science Meeting, to showcase NCI-supported translational research and expand the range of interactions between NCI-supported investigators and mechanisms, were achieved through the active poster discussion sessions. The meeting introduced the translational research community to the TRWG Pathways to Clinical Goals, which are envisioned to be useful for research project management, research program management, coordination of research efforts, and teaching/communication purposes. The participants were exposed to the information considered necessary to evaluate and prioritize translational research opportunities through the ñTranslational Research Opportunity: Information Guides.ò The Abstracts presented at the meeting and the example Translational Research Opportunities will be used by NCI to determine if there are compelling translational research projects that warrant acceleration. The development of a prioritization process, funding mechanism, and project management strategies are pending final NCI approval and resource allocation.

APPENDIX

EXAMPLES OF TRANSLATIONAL RESEARCH OPPORTUNITIES

NCI Translational Science Meeting

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Agent—Targeting the Wnt pathway

Presented by Stephen Baylin, Johns Hopkins University School of Medicine

Credentialing: Detailed characterization of stem cell regulatory networks active in cancer will likely yield powerful diagnostic and prognostic markers as well as attractive targets for therapeutic intervention. The Wnt pathway represents one potential target for intervention. There is an extensive body of literature suggesting the Wnt pathway may contribute to a number of cancer types. Although there has been little effort to develop clinical agents to interfere with the pathway, there are several members of the pathway that may be amenable to pharmacological manipulation, including the Wnt ligands, β-catenin, and Axin.

Creation of modality: It has been hypothesized that Wnt-mediated degradation of Axin is critical for activation of the Wnt pathway. Experiments were designed to determine whether a small molecule that blocks Axin degradation could decrease intracellular levels of β-catenin and prevent Wnt pathway signaling. A high-throughput assay that recapitulates activation of the canonical Wnt pathway in *Xenopus* egg extracts was developed and used to screen drug libraries for regulators of β-catenin and Axin turnover. This approach identified 19 lead compounds, including VU-WS30, which has already been approved by the Food and Drug Administration (FDA) for use as an anti-helminth agent.

Supporting tools: The effect of VU-WS30 on Wnt signaling has been tested in several non-mammalian systems. The compound blocks induction of secondary axis formation in *Xenopus* embryos in a concentration-dependent manner, indicating inhibition of the Wnt pathway. It also alters vulval and cuticle formation in *C. elegans* and *D. melanogaster*, respectively, demonstrating that its molecular target is conserved among metazoans. Finally, VU-WS30 was shown to inhibit Axin degradation in *Xenopus* extracts and cell culture, suggesting that it downregulates the canonical Wnt pathway by potentiating the function of Axin.

VU-WS30 has also been used in cell culture and animal models. In cultured mammalian cells, the compound inhibits transcription of Wnt target genes and decreases cytoplasmic levels of β-catenin. VU-WS30 is also able to inhibit β-catenin-induced proliferation of breast and colon cancer cell lines and synergizes with 5-fluorouracil to induce apoptosis of cancer cells. Actin staining of these cells reveals an alteration in cellular morphology suggestive of a reversal of an epithelial-mesenchymal transition. The compound is also being studied in mouse models.

Preclinical development: VU-WS30 has been shown to interfere with Wnt signaling. However, the molecule is of limited utility as a cancer therapy due to the fact that it was originally designed as an anti-helminth agent and, thus, has limited systemic access. In order to further develop this therapeutic strategy, this lead compound must be modified, and the appropriate pharmacokinetic and toxicity analyses performed.

Clinical trials: Clinical trials could proceed in colorectal cancer based on evidence of Wnt involvement in this cancer type.

Biospecimen-Based Assessment Modality—Biomarkers in DCIS Breast Cancer

Presented by Joe Gray, University of California Lawrence Berkeley National Laboratory

Credentialing: As a result of increased mammographic screening, the rate of diagnosis of ductal carcinoma *in situ* (DCIS) is increasing. Although only 5-10% of DCIS lesions diagnosed will progress to invasive cancer within 5 years, most women are treated very aggressively. Molecular markers are needed so that treatment can be tailored to risk.

One potential biomarker is 14-3-3ζ. Upregulation of this protein results in decreased levels of p53 and increased levels of TGFβ. It also appears to drive epithelial-mesenchymal transition and confer resistance to *anoikis in vitro*. Levels of 14-3-3ζ begin increasing in atypical ductal hyperplasia and upregulation is evident in more than 40% of advanced breast cancer. High levels of 14-3-3ζ correlate with poor patient survival.

The retinoblastoma pathway, which has been identified as a key stress response regulator in human mammary epithelial cells, is another potential biomarker. Increased activity of the retinoblastoma pathway, indicated by high levels of Ki67, p16, and/or COX2, is associated with basal-like breast cancers. Elevated levels of these proteins are indicative of abnormal response to cellular stress and correlate with recurrence of DCIS as invasive cancer.

Creation of modality: There are established immunohistochemical techniques for evaluation of 14-3-3ζ, Ki67, p16, and COX2; however, the requirement for tissue to study these proteins is a drawback. Retrospective validation studies have been carried out for the stress response biomarkers, but independent laboratory validation is still needed. A study is being initiated to retrospectively validate 14-3-3ζ. These studies are hindered by lack of DCIS tissue samples with 15-year follow-up data. Furthermore, tissue samples that are available are small and can only be used to analyze a few markers.

Preclinical development: Prospective validation studies are needed to evaluate these and other biomarkers. These studies need to be initiated as soon as possible because it will take 15 years for them to yield results.

Supporting tools: There are a few small cohorts for these types of studies, but large numbers of patients are needed to generate meaningful results.

Clinical trials: The large-scale, long-term effort required to carry out human studies on these biomarkers should be driven by large national consortia and is an ideal opportunity for NCI support.

Image-Based Assessment Modality—PSCA Imaging of Prostate Cancer

Presented by Sam Gambhir, Stanford University

Credentialing: The prostate stem cell antigen (PSCA) is a prostate-specific glycoprotein that is overexpressed in prostate cancer, including androgen-independent prostate cancer. Although the body of literature describing PSCA in prostate cancer is still expanding, current evidence sufficiently warrants pursuing the protein as a target for imaging. Sensitivity for detecting PSCA will depend on the imaging technique used and levels of the protein in cancer cells. PSCA is expressed in normal prostate cells as well as cancer cells; therefore, it will likely be useful for imaging advanced disease rather than tumors in the prostate bed. Imaging of PSCA could be used in conjunction with 99mTc-MDP or F-bone scans for staging and monitoring response of advanced (disseminated) disease to therapy.

Creation of modality: Engineered antibody fragments are currently being developed to enable imaging of PSCA. These antibody fragments can be labeled with different molecules to permit detection with various existing imaging platforms (e.g., PET, SPECT, optical). Pharmacokinetic analyses and dosimetry will need to be performed for each labeled antibody fragment.

Supporting tools: A number of assays and supporting tools, including tracer kinetic models and physical phantoms, will be needed to develop antibody fragments to detect PSCA. Processes and instrumentation must be developed to automate synthesis and radiolabeling of the agents, and a mechanism for distributing the agent to multiple institutions will also be needed.

Preclinical development: In order to carry out preclinical development of an anti-PSCA antibody fragment, issues related to humanizing the antibody fragments must be resolved and GMP/GLP production established. Preclinical models will be needed for dosimetry and toxicity studies, and Phase I studies in humans will also be needed to assess dosimetry, toxicity, optimal imaging times, and biodistribution. Studies in humans will require filing an Investigational New Drug application with the FDA, but equipment approvals will not be necessary since the agent will be designed for use with existing platforms. Studies should be discussed with local Radioactive Drug Research Committees as necessary.

Clinical trials: Early-phase clinical trials could use PET-CT imaging to evaluate an anti-PSCA antibody fragment in patients with metastatic prostate cancer. PSCA may also be a valuable biomarker for pancreatic cancer, so this population could also be considered for inclusion in clinical trials. PSCA imaging could be compared with existing strategies, including bone scanning and FDG PET-CT. Studies should also be designed to gain insight into the limitations of using PSCA as a biomarker for tumors in the prostate bed. Importantly, imaging protocols and image acquisition must be standardized across study sites. The American College of Radiology Imaging Network should be involved in coordinating multicenter trials.

Immune Response Modifiers—WT-1 Vaccine for Minimal Residual Disease in WT-1-positive Acute Myeloid Leukemia and Ovarian Cancer

Presented by Martin Cheever, Fred Hutchinson Cancer Research Center

Credentialing: There are many credentialed targets for cancer vaccines, the ultimate targeted therapy. One potential antigen for vaccine development is WT1. WT1 is oncogenic and is expressed, often at high levels, by the tumors of many cancer patients, including those with acute myeloid leukemia and ovarian cancer. It is also thought that WT1 is expressed by stem cells. Clinical trials have shown that WT1 is immunogenic and peptide-based vaccines have induced regressions in several patients with acute myelogenous leukemia. The protein has multiple T cell epitopes that could be targeted by the immune system and thus longer vaccine constructs appropriate for most if not all patients are possible.

Creation of modality: In addition to a target antigen, cancer vaccines must be associated with a formulation, e.g., delivery vehicle, regimen, collaborative immune response modifiers. Decisions must be made related to the antigen, the formulation and the regimen that hold the highest potential. Potential formulations for a vaccine targeting WT1 include a prime/boost approach and use of adjuvants (e.g., CpG, MPL). Immune response modifiers that could be used in the regimen in conjunction with the vaccine include IL-7 and anti-PD1. IL-7 is a homeostatic T cell growth factor. Clinical trials have validated that injection of IL-7 can increase the absolute number of naïve T cells in normal individuals. Anti-PD-1 is an inhibitor of T cell checkpoint blockade and is capable of greatly increasing the level of immune T cell expansion to antigen stimulation. Both IL-7 and anti-PD-1 are immune response modifier agents from a list of NCI prioritized agents with the potential to cure patients with cancer, if they were available for broad testing and development with cancer vaccines.

Supporting tools: WT1 vaccines and proposed collaborative immune response modifiers have been separately validated in animal models and clinical trials. Mice are appropriate models for study of WT1 vaccines as their WT1 is essentially identical to human WT1 with a similar distribution and level of expression. Like humans, mice exhibit substantial immunologic tolerance to WT1. However, despite immune tolerance, it is possible to elicit immune responses to mouse WT1 in mice. Supporting tools required to evaluate response to a WT1 vaccine include biomarkers to identify patient cohorts for clinical studies, assays to measure immune response and clinical response to the vaccine. Patients could be selected for clinical trials based on whether their tumors express WT1. WT1 expression could be assessed via RT-PCR or immunohistochemistry. The pharmacodynamics of a WT1 vaccine could be studied using assays to measure direct immune response (e.g., T-cell responses, antibody responses) and spread immune response. Anti-tumor responses can be measured by determining changes in WT1 in peripheral blood and bone marrow using RT-PCR. Imaging could be used to determine whether activated T cells are present at the tumor site.

Preclinical development: Preclinical development will require manufacturing of overlapping WT1 peptides or gene-based vaccines as well as necessary adjuvants and immune modulators. All of these components can be manufactured relatively easily or have been manufactured, but this does not guarantee their availability.

Clinical trials: As the WT1 vaccine progresses to clinical trials, iterative Phase I trials that incorporate immunity, tumor marker, and molecular imaging endpoints should be conducted. Various combinations of vaccine, adjuvants, and immune modulators should be tested. Phase II trials should only commence after a predefined level of immunity has been achieved in Phase I trials. Therapeutic cancer vaccine trials would best be done with a collaborative network of a broad range immunology, immunotherapy and clinical trial experts.

Interventive Devices—Development, Optimization, and Validation of Irreversible Electroporation; Image Planning, Guidance, and Monitoring for the Treatment of Hepatocellular Carcinoma

Presented by Gary Dorfman, Weill Cornell Medical College

Credentialing: Irreversible electroporation (IRE) creates microscopic pores in the cell membrane through application of millisecond, high-voltage electrical pulses. Cells are subsequently unable to maintain homeostasis and undergo apoptosis over a 24-hour period. IRE is not susceptible to the heat sink effect and has a predictable ablation zone. Since IRE purportedly preserves underlying collagenous structures, it can theoretically be used to target tissue adjacent to blood vessels, nerves, intestines, and other tissue.

There is an emerging clinical need for less-invasive therapies. Treatments for most solid tumors currently involve surgery and systemic therapy, but patients would likely prefer targeted, local/regional therapy to major surgery. Patients with hepatocellular carcinoma may be good candidates for local/regional therapy since many of these tumors cannot be curatively excised.

Creation of modality: IRE devices are currently available for clinical use, but are not fully optimized and have not yet been integrated with imaging. The proposed new modality would include IRE tightly integrated with imaging for planning, guidance, and monitoring. Ablations in phantoms, isolated organ preparations, and animals will be necessary to validate claims of safety to adjacent structures, optimize performance, and evaluate imaging options for tight integration.

Supporting tools: Supporting tools will be needed for both monitoring of response and cohort identification. It will likely be necessary to use multiple modalities to gather information about both anatomic and physiologic parameters; importantly, evaluation should address both affected and residual viable tumor cells. It may be possible to use the ApoSense assay to visualize early commitment to apoptotic death. Magnetic resonance imaging could be used to observe alterations in pH as well as diffusion and cell permeability. Nanoparticle agents may also be useful for assessing cell membrane porosity. Visualization of residual viable tumor cells will be challenging but may be achievable if a biomarker for hepatocellular carcinoma cells can be identified and imaged. Assessment modalities will need to be developed to identify patient cohorts. Biospecimen repositories and appropriate sampling tools will be helpful in this regard.

Preclinical development: Preclinical development of this modality will require partnership between the IRE and imaging industries, which will pose intellectual property issues. It may be necessary to carry out some studies in humans during this phase of development to confirm safety and optimize system performance prior to assessing whether the desired target is affected.

Clinical trials: Phase I clinical trials should entail ñdose-escalationò of IRE for assessment of safety in combination with image-based planning, guidance, and monitoring. Secondary endpoints could include validation of biomarkers for detection of residual tumor. Trials could involve ablation followed by surgical resection or biological therapy. It would be informative to collect tissue before and after ablation so that correlative studies can be performed.

Lifestyle Alteration—Diet (and Exercise) Intervention to Reduce Breast Cancer Risk

Presented by Stephen Barnes, University of Alabama at Birmingham

Credentialing: Cancer rates differ between countries, and migration provides an interesting scenario for investigating the reasons for observed variances. Women who emigrate from Southeast Asia to the United States as adults maintain a similar breast cancer risk as counterparts in their country of origin; however, Asian girls who emigrate earlier in life exhibit risk similar to U.S-born women. Also, it has been shown that women exposed to atomic radiation between the ages of 15 and 20 have higher rates of breast cancer than women exposed at other ages. Together, these data suggest that events occurring during puberty significantly influence breast cancer risk.

Reducing the incidence of breast cancer, one of the most commonly diagnosed cancers, would have tremendous social and economic impact. Diet and exercise interventions are also appealing because they may also reduce the incidence of other maladies, such as cardiovascular disease. Diet and exercise interventions are feasible, particularly among children.

Creation of modality: One approach for examining how lifestyle impinges on breast cancer risk is to determine how diet and exercise influence gene expression, a phenomenon that has been documented in animal models. This type of experiment must be carried out as an observational rather than an interventive study. For example, studies could be done on pairs of athlete/nonathlete sisters who will have similar genes and environmental exposures but different levels of exercise.

Supporting tools: Several potential populations could be studied. The NIH National Childrenôs Study has invested $3.2 billion to follow a cohort of children from birth to age 21. The Komen Foundation is generating a biospecimen repository of tissue from healthy women. Increasing numbers of outlets for girlsôsports will likely provide valuable opportunities to carry out these types of studies. In order to identify a cohort of pubertal girls, it will be necessary to identify and validate markers of puberty that can be easily measured in urine or blood. Supporting tools may also be needed to measure genetic/epigenetic changes that may be correlated with breast cancer risk (e.g., assay to measure histone acetylation).

Preclinical development: Preclinical studies of the impact of lifestyle on breast cancer can be carried out in mice, including mice treated with carcinogens. It has been shown that the effect of mammary carcinogens in mice can be age dependent. Once candidate genetic/epigenetic changes are identified through observational studies in humans, the effects of these changes can be more thoroughly dissected in mouse models. It may also be possible to use imaging to track the changes of specific proteins in live animals.

Clinical trials: Retrospective and observational trials have examined the relationship between lifestyle and breast cancer risk, but systematic intervention trials have not yet been performed and will be required.